MASTER'S THESIS

Acute immune response of elite rugby union players to Super 15 Match-play

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Acute immune response of elite rugby union players to Super 15 Match-play.

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Declaration

This thesis is submitted to Bond University to fulfil the requirements of the Master's Degree by Research. The material in this thesis does not contain previous material that has been submitted or published in universities or other institutions to the best of my knowledge, unless due acknowledgement has been made within the thesis.

Signed: _____

Sean Yoshiura

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Abstract

The present study observed the immunological response to Super 15 match-play by analyzing weekly work load, match statistics, and [s-IgA] of elite rugby union players (n = 23, age 24.8 ± 2.9 years; stature 185.6 ± 6.8 cm; weight 100.7 ± 11.2 kg, Super 15 career of 56.3 ± 24.7 rugby games) competing in three Super 15 rugby matches (Match 10, Match 12 and Match 15)**.** Saliva samples (0.5 ml) were collected at 32 hours pre match, 1 hour post match play and 32 hours post match play, using the Individual Profiling device (IPRO) (Oxfordshire, UK) to determine the [s-IgA]. Training and game loads (RPE x Duration) were recorded to calculate weekly workloads across the Super Rugby season.

A substantial increase in [s-IgA] was observed pre 32 hour match play and 1 hour post match play in Match 10 for backs (45.8 ± 22.6 %, ES 1.78 ± 0.96) and forwards (49.3 ± 25.8 %, ES 1.29 ± 0.74), and pre 32 hour match play and 32 hours post match in Match 10 (23.1 ± 50.8 %, ES 0.98 ± 1.93) for backs and forwards (50.9 ± 38.9 %, ES 1.32 ± 1.06) and Match 12 for backs (54.2 ± 45.2 %, ES 1.06 ± 0.92) and forwards (40.3 ± 32.6 %, ES 0.89 ± 0.74) compared to all other time points. Acute weekly changes (week to week changes) in physical loads found substantial decreases during Match 10 for backs (-26.1 ± 12.1 %, ES -2.36 ± 0.92) and forwards (-37.5 ± 22.3%, ES -2.21 ± 0.95) and Match 15 for backs (-19.1 ± 37.8%, ES -0.89 ± 1.35) and forwards (-22.0 ± 30.0%, ES -1.10 ± 1.17), and substantial increases during Match 12 in both backs (23.8 ± 37.8%, ES 0.50 ± 0.75) and forwards (14.8 ± 17.2%, ES 0.53 ± 0.61). Accumulated changes in (training stress balance) also found same trends seen in acute weekly changes, where there were substantial decreases during Match 10 and Match 15, and substantial increases during Match 12 in backs and forwards.

The results of this study indicate inconsistencies in the changes in [s-IgA] across multi-match analysis, which highlight individual variation in response to the demands of rugby match play. The possible mechanism for the substantial increase in [s-IgA] post rugby match play in Match 10 and Match 12 remains unclear, but it could be attributed to the immune rebounding effect from the physiological disruptions seen in rugby match play. The variation in response to the physiological demands of Super Rugby match play highlight the importance of monitoring players on an individual basis to determine whether rugby union players are able to cope with the demands of rugby match play or are at risk of injury or illness. The present study also found significant increases in [s-IgA] during recovery periods (32 hours post-match play), which suggests the host immunity may not fully restore to its baseline values.

The findings of this study also found decreases in weekly training load could potentially have an effect in minimizing the suppression of immune function. As a result, strategic periodization in player loading needs to be considered to reduce the risk of suppression in the immune function and subsequent risk of contracting URTI.

The practical application of the present research suggests the importance in combining athlete monitoring methods that include s-IgA and the analysis of player work load to determine the immunological response of elite rugby union players to the physical workloads and demands of Super Rugby.

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List of Symbols / Abbreviations

DALT – Duct-associated Lymphoid Tissue

ELISA – Enzyme Linked Immunosorbent Assay

Fab – Fragment Antigen-Binding

Fc – Fragment crystallisable

GPS – Global Positioning System

HPA axis – Hypothalamic Pituitary Adrenal axis

IgA – Immunoglobulin A

IgG – Immunoglobulin G

IgM – Immunoglobulin M

IPRO – Individual Profiling Device

LFD – Lateral Flow Device

MALT – Mucosal-Associated Lymphoid Tissue

OFC – Oral Fluid Collector

pIgR – Polymeric Immunoglobulin receptor

RU – Rugby Union

s-IgA – Salivary Immunoglobulin A

[s-IgA] – Salivary Immunoglobulin A Concentration

SC – Secretory Component

URTI – Upper Respiratory Tract Infection

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Chapter One: Introduction

Rugby Union (RU) is a competitive team sport that involves high intensity activities that include acceleration and deceleration efforts, running, sprinting, rucking and repeated heavy impact collisions between two teams to ultimately score points to attain victory [3, 6, 70]. Rugby Union is played worldwide, with the Super 15 rugby competition comprising the elite club level of competition in the southern hemisphere and includes representation from professional rugby franchises located in Australia, New Zealand and South Africa [70]. The Super 15 competition is played annually from February to August with teams travelling internationally to participate in the regular season "home and away" competition [70]. The season consists of 20 home and away matches followed by three weeks of finals. Unlike other professional rugby competitions around the world, the Super 15 competition presents players, coaches and performance staff with unique logistical and performance challenges that influence individual immunological profile and recovery over the course of the season. These unique challenges may include international travel and flight conditions lasting up to thirteen hours, environmental conditions, for example, variations in humidity, altitude, training and playing surfaces, and the potential influence of cumulative fatigue associated with weekly regular season matches played approximately seven days apart. The diverse logistical considerations associated with participating in the Super 15 match play places considerable physiological demands on players such as game-induced immunological and endocrinological disturbances [6], muscle damage [53], neuromuscular fatigue and mood disturbances [9]. The physiological demands of rugby match play, and individual adaptation considerations challenge the strength and conditioning staff to maintain player's fitness level and reduce the risk of overtraining, illness and injuries throughout the season.

To investigate the physiological demands of rugby union match-play, previous studies [3 - 7] have incorporated a range of qualitative and quantitative measures to gain a greater insight into the positional demands of professional rugby union players at Super 15 and international level. Methods in collating quantitative and qualitative analyses of rugby match play include match activity profiles through time-motion analysis and portable global positioning system (GPS) and integrated accelerometry [3 - 7], and pre and post-match neuromuscular [9] and biochemical analyses [6]. However, no study has investigated the effects of salivary immunoglobulin A (s-IgA) in response to the physical demands of rugby match play.

Portable monitoring technology, using GPS and integrated accelerometry [70], and time-motion analyses [3] have enabled investigators to quantify movement profiles in rugby players during competitive match-play [3 - 7]. Time motion [3] and GPS and integrated accelerometry [70] analyses of Super rugby match-play have highlighted the ongoing evolution of the demands of competition, such as players covering greater total distances [3, 70] and are involved in more high intensity activities and sprint more frequently [3, 70]. Positional differences also exist in rugby match play, where backs cover greater total distance, high-speed distance and quantity of accelerations

compared to forwards, who display a higher collision profile [3 - 6, 70] during competition.

The characteristics of rugby match-play, such as heavy collisions, repeat accelerations, and high intensity distance runs result in tissue trauma [53, 71] that could induce pro-inflammatory disturbances in the human body [6, 53, 71]. Biochemical analyses of international rugby match play by Cunniffe et al. [6] found elevations in acute inflammatory markers and suppression in immune markers post rugby match play. Host immune protection in elite rugby union players was also compromised for up to 38 hours during the recovery period [6]. Currently, no studies have investigated the immune response of elite rugby players 38 hours post Super Rugby match play [91]. Further research is thus required to investigate whether Super 15 rugby match play produces a similar immunological response to international rugby match play, which may influence the vulnerability of the immune system against airborne and viral infections during recovery periods.

The analysis of immunological markers can provide strength and conditioning coaches with a more comprehensive understanding of the demands of competition and subsequent recovery patterns, and contribute to the quantification of an athlete's immune system and, subsequently, their risk of upper respiratory infection [29]. Salivary analysis of immunological markers has been shown to be a non-invasive method of determining an athlete's risk of contracting an upper respiratory tract infection (URTI), which may be the result of immunological suppression in response to training and match play demands as observed in American football [39], rugby [15, 44] and soccer [16, 72]. Salivary immunoglobulin A has recently emerged as a reliable non-invasive measure to quantify the immunological capacity of an individual in response to acute [4] and chronic bouts [15] of training [15] or rugby match play [4]. Salivary immunoglobulin A plays a crucial role in the first line of oral mucosal defence against bacterial intrusion along the epithelial surface by neutralising antigens and external pathogens entering in the mucosal surface [33].

The acute response of s-IgA to prolonged strenuous bouts of exercise remain unclear. Previous literature has found s-IgA to increase [11, 16, 40] decrease [41, 42, 43, 14, 48], or have no significant changes [44] in response to either prolonged or high intensity exercises such as that undertaken by rugby players during competition. The inconsistency of research [34, 40, 44, 48] investigating the response of s-IgA to running [34], basketball [40], triathlon [48] and rugby match play [44] demonstrate that the acute s-IgA response may vary depending on the individual's physical work capacity, the intensity, duration, frequency of exercise [25] and the type of sport conducted. In relation to rugby union, Koch et al. [44] investigated the acute response of rugby union matches in collegiate rugby union players [44]. Koch et al. [44] found no significant differences ($p > 0.05$) in [s-IgA] and s-IgA secretion rate pre and post rugby match play in collegiate rugby players [44]. Koch et al. [44] highlighted the variations in immune response are attributed to within-subject variation of game demand, the influence of fluid intake, sweat rate and

the small sample size in the study.

Previous longitudinal studies in soccer [75], American Football [25] and rugby [15] have proposed that during intense periods of competition, decreases in s-IgA concentration ([s-IgA]) has been observed, which may lead to an increased risk of URTI among athletes. An increase in URTI risk may be attributed to a compromised mucosal defence system due to reduced [s-IgA] levels [15, 25, 75]. The decrease in host immune protection may allow antigens to enter along the epithelial surface, where the antigen will replicate, and disrupt the epithelial barrier by increasing vascular leakage and mucosal secretion [15, 25, 75, 76]. In the sport of rugby union, a longitudinal study by Cunniffe et al. [15] investigated the immune response of elite rugby players to the physical demands of European rugby competition. Cunniffe et al. [15] found similar findings to the studies by Mortatti et al. [75] (soccer) and Fahlman et al. [39] (American Football), where the highest incidence of URTI coincide with lower [s-IgA] in rugby union players during intense periods of competition. Cunniffe et al. [15] concluded the variances in training load may induce further physiological stress in rugby union players, which will reduce host immune function and predispose players to URTI. The impact of weekly high intense training sessions and match play of a Super Rugby season to the immune system in elite rugby union players are yet to be investigated and, as such, the response is unknown.

Saliva analyses are a non-invasive, valid and effective tool in assessing the hormonal, immunological and endocrinological profiles of rugby union players in a sporting environment [15, 44]. The current "gold standard" of bio-sensory immunoassay is the Enzyme Linked Immunosorbent Assay (ELISA) method, which is utilised to quantify and analyze the salivary analyte concentrations within a collected sample [11, 16, 23, 25, 39]. The ELISA method has previously been used in the sport of rugby to determine the s-IgA response of rugby union players to training [15] and match play [44] effect. Cunniffe et al. [15, 44] found suppression in [s-IgA] of elite rugby union player during intense training periods of competition[44] and in post international match play [44]. The limitation of the ELISA method, such as lengthy experimental procedures, logistical considerations in purchasing equipment, and the high level of expertise required to collate the data, makes it difficult to implement within a professional sporting environment. Time of analysis to collect data and provide real time information on the [s-IgA] to high performance staff can be addressed via the use of a new technology such as the Individual Profiling (IPRO) device. The IPRO is a portable saliva analysis device, consisting of an immunochromatographic test strip, reagent buffer and immunochromatographic test strip that enables analyses of salivary analyte concentration such as s-IgA [46]. Validity and reliability tests of the IPRO device by Coad et al. [67] were conducted on recreational active males (n = 12) and females (n = 13). Coad et al. [67] found the use of the IPRO device to be a reliable ($r = 0.89$, $p<0.001$ and CV = 9.40%) and valid ($r=0.93$, $p<0.001$) substitute for the ELISA method to

measure [s-IgA] in professional rugby union players following match-play. Coad et al. [67] also investigated the Bland Altman analysis to determine the measures of agreement between the IPRO and ELISA for s-IgA. The

Bland Altman plot found unbiased agreement between IPRO and ELISA for sIgA with a mean difference of 1.2 µg/mL ± 1.96 SD [67]. The IPRO device's portability and time efficiency (approximately seven minutes) in obtaining a quantitative analysis of salivary analytes [46] provides a more suitable tool for real time salivary analyses when travelling overseas such as South Africa and New Zealand during the Super Rugby competition.

The aim of the present research was to determine the immunological response of elite rugby union players to the physical demands of Super 15 Rugby match-play and whether variances in week to week workloads in Super Rugby competition will influence the immune response of elite rugby union players to rugby match play. The present research provides a greater insight with respect to the individual s-IgA response to Super 15 match-play, a well-established international level of competition across Australia, South Africa and New Zealand, which should lead to further understanding of the physiological demands of competition and the subsequent prescription of weekly training load and recovery period post-match.

Research Questions

1) Do the physiological demands of Super 15 match play result in suppressed immune system in elite rugby union players?

2) Do elite rugby union players experience a prolonged immunological suppression in the days after match play?

3) Do changes in week to week workloads in Super Rugby competition influence the immune response of elite rugby union players to rugby match play?

Chapter Two: Literature Review

2.1 Physiological Demands of Rugby Union

The physiological demands of elite rugby union players during match-play have previously been identified using time and motion analysis via computer-based tracking in national match-play [3] and GPS and integrated accelerometry in national [6, 70], international [5] and sub-elite [7] competition. Global Positioning System (GPS) and integrated accelerometry analyses suggest positional demands elicit different physiological loads during matches in Super 15 competition [70], English Premiership competition [4], Spanish Elite rugby union fixture [8] and in international rugby fixtures [5, 6]. Movement characteristics of rugby match-play, using GPS and integrated accelerometry demonstrate backs travel greater total distances and increased distances at high intensity such as sprinting or striding, compared to forwards in rugby [4 - 6, 8]. Accelerometry profiles on impact associated with collisions during attacking and defensive play also indicate that forwards experienced greater total impacts and heavy impact (above 7G) collisions during match-play compared to backs [5]. The discrepancies reported between the physiological demands of match play experienced by backs in comparison to forwards demonstrates the importance of monitoring positional workloads in rugby union.

The physical demands of Super 15 rugby have previously been investigated [70, 3]. McLellan et al. [70] and Austin et al. [3] were able to highlight the increases in total high-intensity activities, sprint frequency, and work to rest ratios at Super 14 and Super 15 level when compared to Super 12 competition [3]. McLellan et al. [70] investigated match-play performance characteristics among Super 15 rugby players using GPS and integrated accelerometry and found Super Rugby union players must be able to sustain repeated bouts of high-intensity activity (repeated sprint and high intensity running) and endure repeated and frequent blunt force trauma elicited by high impact collisions (> 7 G) [70] during a Super 15 match play. Positional demands must also be taken into consideration as backs were found to have greater total relative mean distance, high intense running and sprint activities compared to the forwards [70]. McLellan et al. [70] also highlights the use of match video analysis to quantify non-locomotor high intensity activities such as pulling, pushing, and tackling in Super Rugby match play. No studies, however, have reported the movement characteristics and variation that may exist between the physiological demands of training and match play at Super 15 level. Further studies need to be conducted to determine whether the accumulated fatigue from rugby training will influence the immunological response to Super 15 match play. The present study will investigate whether the acute and chronic changes in weekly loads during the Super Rugby competition will influence the immunological response to Super 15 match play.

2.2 Physiological Response to Rugby Union Match-Play

Cunniffe et al. [6], Takahashi et al. [14] and West et al. [9] investigated the endocrinological [6,14], immunological [6] and neuromuscular [9] responses to elite rugby union match-play. Cunniffe and colleagues [6] found the physiological demands of international rugby match play in elite rugby union players transiently influenced a number of immunological parameters such as T lymphocytes, NK cells and bacteria-stimulated neutrophil degranulation activity at immediately post-match, 14 hours post-match and 38 hours post-match. Cunniffe et al. [6] also found immunological parameters did not return to baseline values within 38 hours post-match. Cunniffe et al. [6] stated that due to the delay in immunological parameters to return to baseline values, elite rugby union players require an adequate recovery period of 38 hours post rugby match play. Takahashi et al. [14] investigated the response of neutrophil activity in rugby seven players over two successive rugby seven matches. The study by Takahashi et al. [14] found no significant ($p > 0.05$) differences in neutrophil activity after the initial match, but found significant decrease ($p < 0.05$) in neutrophil activity after the second consecutive rugby match play[14]. Takahashi et al. [14] proposed the suppression in neutrophil activity was potentially due to accumulative fatigue attained from the initial game, which subsequently impacted on the immune response during the second match [14]. Previous studies by Cunniffe et al. [6] and Takahashi et al. [14] demonstrated a suppression in immune function in rugby union players in response to the physiological demands of rugby match play at international and rugby sevens format.

The present study will investigate the s-IgA response in a Super 15 rugby match, which will impose different intensity and physiological demands compared to international and rugby sevens format. An analysis of the s-IgA response to Super 15 rugby match-play will provide a greater understanding of the acute effects on the immune system in response to the competition demands of Super Rugby and provide coaches and sports scientists with further information regarding the chronic response of s-IgA over the course of a regular season period of Super 15 rugby competition.

2.3 Immune Function and Exercise

The relationship between exercise and immune function does not exhibit a linear relationship [10], as different modes of exercise can elicit various responses of the immune system [10]. As observed in Figure 1, Nieman [10] proposed that the relationship between immune function and exercise is illustrated as a "J-Curve", dependent on the relative intensity, duration and frequency of exercise.

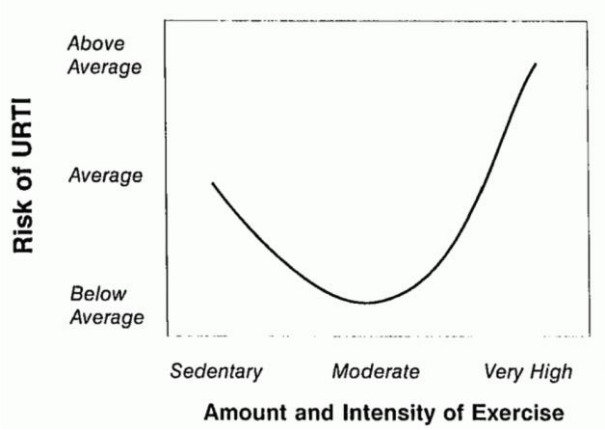

Figure 1. The J-Curve Model, illustrating the relationship between the risk of URTI and the amount and intensity of exercise. Adapted from Nieman [10].

The acute response of sedentary subjects to moderate levels of exercise has been found to either have no effect or enhance immune function in sedentary and athletic populations [10]. The acute effects of prolonged high intensity exercise on immune function has shown to be suppressive, particularly among athletic and sedentary populations[10]. Immunological suppression occurs when exercises are conducted in excess of 1.5 hours and of moderate to high intensity (> 55 – 75 % of VO_{2max}) [18, 21, 22, 23, 25] such as that experienced by athletes during sports such as soccer [72], and rugby union [6, 44]. The theory associated with the acute immune suppression may be explained by the phenomenon known as the "open window theory" [10]. When athletic and sedentary populations complete intermittent and continuous exercise, the inflammatory system is activated to initiate the repair phase of damaged muscles [26]. Nieman [26] suggests that the inflammation may cause the immune system to increase its involvement in the inflammatory response to heavy exertion of exercise and reduce the protection against infections such as URTI. During the inflammation process, s-IgA activates FcαRI-mediated inhibitory function [62]. The activation of the FcαRI complex causes the inhibition of inflammatory pathways to occur such as IgG-mediated phagocytosis in monocytes [62]. The shift towards the initiation of the inflammatory response causes the immune cells to be suppressed, and leaves the host immune protection vulnerable [62]. Decrease in host immune protection allows antigens and other environmental pathogens to enter the epithelial surface [50]. Once antigens have entered the cell, the antigens will multiply and initiate the inflammatory response to occur via the binding of inflammatory cells such as cytokines and chemokine [50].

Pyne and Gleeson [27] observed the effects on s-IgA in swimmers across a seven-month training camp, and found athletes with lower pre-season s-IgA were predisposed to contracting URTI during the seven-month training period. Pyne and Gleeson [27] suggest that a URTI will impede an athlete's performance, or potentially force athletes to miss competition and training. Cunniffe, et al. [15] investigated the immunological response in elite rugby union players to demands of English rugby union competition, suggested that contracting URTI is particularly detrimental in team sports where absence of players will impact on team resources to perform to their desired match play, which will cause setbacks to team performance. The restoration of immunological parameters such as T lymphocytes, NK cells and bacteria-stimulated neutrophil degranulation to their baseline values may take up to 38 hours post-match among rugby union players, and may indicate a period of immunosuppression and increased risk of URTI subsequent to competition [6]. Suppression in immune function such as that demonstrated via a reduction in s-IgA over 24 hours can predispose athletes to viral infection post-intense exercise or match play in swimming, [11, 18], marathon [41], futsal [42] swimming [43], and triathlon [48]. The present study will investigate the pattern of s-IgA in response to RU competition, which may provide high performance staff with an increased understanding of the potential risk of contracting viral infections, athletes and coaches will be able to strategically plan modified training and recovery protocols in response to post-competition such as a rugby union match.

2.4 Salivary Immunoglobulin A – Structure and Synthesis

Salivary Immunoglobulin A is an antibody that plays a crucial role in the first line of defence against microbial adhesion along the mucosal surface of epithelial cells [28]. Salivary Immunoglobulin A is also the predominant antibody that is externally secreted in saliva [17]. Walsh et al. [29] reviewed previous literature investigating the effects of immunological parameters such as neutrophil activity, natural killer cell activity, T and B Cell proliferation, and s-IgA in response to exercises such as cycling and marathon [29]. In comparison to other immunological parameters such as T and B Cell proliferation and neutrophil activity, Walsh et al. [29] state s-IgA is the only immune marker to exhibit a strong correlation with the incidence of URTI during intense training periods or after competition.

Salivary Immunoglobulin A is a glycoprotein made of two IgA monomers, composed of two heavy chains and two light chains [17, 32, 33, 45], as observed in Figure 2. These monomer chains are arranged into two Fragment Antigen Binding (Fab) regions and two fragment crystallizable regions (Fc). The fragment crystallisable (Fc) region plays an important role in mediating interactions with receptors and effector molecules, whereas the Fab region is involved in recognising antigens present along the mucosal surface [17, 32, 33]. Immunoglobulin A in humans has two subclasses: IgA1 and IgA2 [17, 32, 33] and is secreted via the bone marrow, whereas IgA2 is secreted via local plasma cells. The fundamental difference between IgA1 and IgA2 is the absence of a

13-amino acid sequence in the hinge region of IgA2 [17, 32, 33] as observed in Figure 2. The addition of the 13-amino acid sequence in the hinge region,

allows IgA1 to have a greater extension and affinity to detect antigens in comparison to IgA2 [17, 32, 33].

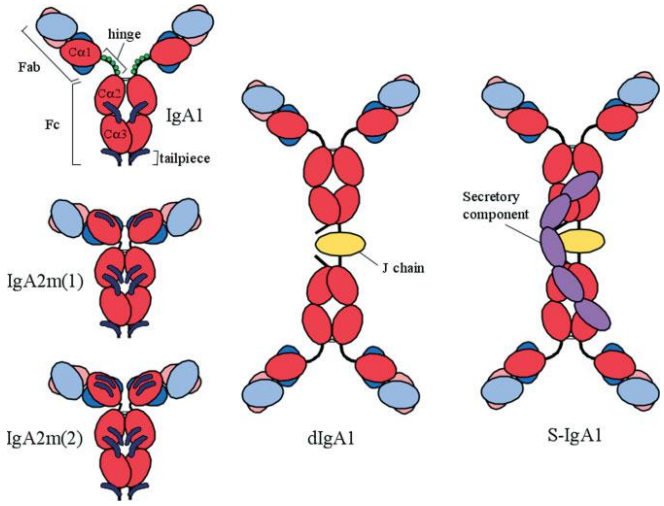

Figure 2. Schematic Diagram of IgA1, IgA2 and s-IgA. Adapted from Woof and Kerr [17].

Salivary Immunoglobulin A is secreted via the T-Lymphocyte dependent B-Cells [32, 35, 37]. Once IgA monomers are secreted, these monomers are covalently bound via a disulphide bridge on the Fc region by two glycoproteins known as the J-Chain and Secretory Component [54], observed in Figure 2. The J-Chain plays a crucial role in differentiating the molecular configuration and maintaining structure of IgA and other antibodies in the mucosal system (namely IgG and IgM) [35]. The Secretory Component of Immunoglobulin A is an extracellular glycoprotein that allows for the s-IgA to be transported to the endothelial surface of the cell to bind to the polymeric immunoglobulin receptor (pIgR) [54]. The activation of pIgR-sIgA complex initiates the transcytosis of s-IgA across the cell through a series of vesicles via the apical recycling endosomes until it reaches the apical surface [36]. The receptor-IgA complex is then transcytosed to the extracellular surface, where it will be cleaving the pIgR-sIgA complex through proteolysis to release s-IgA [37].

The synthesis of s-IgA is stimulated via two antigen driven mechanisms, the Local Response Theory [83] and the Common Mucosal Theory [84]. The Local Response Theory involves the stimulation of lymphoid cells by the detection of oral antigens passing through the ducts of the salivary glands, which will cause proliferation and differentiation of IgA antibodies [83]. Oral antigens enter into the ducts, via natural retrograde flow, and are engulfed the duct-associated lymphoid tissue (DALT) [83]. Antigen recognition by T cells, macrophages and B-cells causes subsequent activation and initiation of s-IgA synthesis [83].

The Common Mucosal Theory involves antigen driven activation of migrated T and B cells in the mucosal-associated lymphoid tissue (MALT) in the salivary gland [84]. The antigen driven activation of T-cells, dendritic cells and T-cell derived cytokines also causes maturation of IgA precursor B-cells to occur [84]. The activated B-cells are transported to the blood circulation from the thoracic duct into the internal membranes of the intestine, lungs and exocrine glands, where terminal differentiation occurs with local T-cells [84]. The cascading effect results in the IgA production by plasma cells in the mucosal and glandular tissue [84].

2.5 Function of Salivary IgA (s-IgA)

Salivary Immunoglobulin A's primary function is to immobilise and neutralise microbial toxins and invasive pathogens to prevent bacterial adhesion across the epithelial surface [34]. If antigens pass through into the cell, s-IgA will also bind to the antigen intracellularly, and travel across the cell via the mediation of the pIgR[34]. The antigen bound IgA complex will be excreted to the lumina where it will be neutralised[34]. Salivary Immunoglobulin A also exhibits an anti-inflammatory role by blocking the binding of IgM and IgG to their respective receptors, which will inhibit complement activation [34].

Salivary [IgA] also has an indirect function to promoting an inflammatory response and activation of phagocytosis via the binding of the Fc**α**RI receptor [34]. Although s-IgA is a poor activator of complement pathways compared to IgM and IgG, s-IgA still binds to specific receptors to activate alternate pathways. The FcαRI receptor is expressed on neutrophils, monocytes, eosinophils, macrophages, interstitial dendritic cells, and Kupffer cells [34]. When the immune response is activated, the binding of the FcαRI and antigen-binding [IgA] complex translates to numerous biological activities [34]. Mechanisms of the FcαRI and antigen-binding [IgA] complex include phagocytosis, respiratory burst induction in neutrophils, cytokine release, antibody-dependent cellular cytotoxicity, superoxide release, and antigen presentation [34].

2.6 Salivary IgA and Exercise

The acute response of s-IgA to intense exercise remains unclear, however previous research [25, 38, 39, 50, 52] has proposed that the [s-IgA] may have an inverse relationship with the incidence of URTI in athletes. Resting [s-IgA] has also been found to be reduced among elite athletes such as swimmers in comparison to the general population [73]. Francis et al. [73] attributes decreases in [s-IgA] levels to highly-intense training loads imposed among elite athletes compared to the sedentary population. The physiological demands of competitive training and competition may increase athlete's predisposition to a high risk of viral infections that is associated with a suppression of host immune protection [5] such as that demonstrated by a reduction in [s-IgA].

Sari-Sarraf et al. [16] and Tharp et al. [40] found post-exercise s-IgA values increased in soccer [16], and basketball matches [40]. In contrast to the work of Sari-Sarraf et al. [16] and Tharp et al. [40], s-IgA decreased post-exercise in marathon [41], futsal [42] swimming [43], and triathlon [48]. Decreased s-IgA levels is strongly related to inadequate recovery and increases in training workload, training volume and match play demands as seen in sports such as rugby union [15]. These decreases in the s-IgA may be due to the altered endocrine response to chronic and high intense exercise [29, 30, 49]. Alterations in the Hypothalamic-Pituitary-Adrenal (HPA) axis will inhibit the synthesis of s-IgA and down regulate pIgR synthesis, which will decrease transcytosis [29, 30]. Decreases in s-IgA synthesis and secretion result in decreases in [s-IgA], which subsequently may increase exposure to external pathogens [29, 30, 49]. The variability in studies mentioned above can also be influenced by multiple factors such as environment [55]; psychological stress [58-61]; flow rate [56, 57]; intensity, frequency and duration of exercise [25]; accumulated fatigue [14]; different methodologies conducted to measure s-IgA [30].

The chronic effects of high intense workloads during the in season and pre-season phase of competitions [1, 2, 12, 15] have demonstrated higher incidences of upper respiratory infections and a decrease in [s-IgA] across numerous sports such as basketball [1], swimming [2, 12, 51], rugby union [15] and American Football [39]. Salivary Immunoglobulin A concentrations below 40mg.L^{-1} [39] has been strongly linked with an increased risk of URTI in American footballers. During intense periods, consecutive bouts of intense exercise impose the immunological system to become suppressed [39]. Without adequate recovery, chronic suppression in s-IgA may ultimately lead to immunological dysfunction, increasing the risk of attaining an URTI [39]. Neville et al. [25] identified decrements in [s-IgA] over three weeks' prior as a key indicator to contracting URTI in sailing. Neville et al. [25] proposed that the negative correlation between [s-IgA] levels and the incidence of URTI may be due to the increased permeability of bacterial adhesion across the epithelial surface due to the immunological dysfunction in s-IgA from consecutive intense bouts of intense exercise.

2.7 Physiological Response to Rugby Union Season

Weekly training and match loads (AU) can be quantified through internal loads [15, 77] by multiplying the Rate of Perceived Exertion, using a Borg Scale CR10 [81], and the duration, or by external loads using GPS and Integrated Accelerometry [91]. Analysing weekly internal loads across the rugby season has been found to be an important marker in identifying elite rugby union players at risk of injury [77] and illness [15]. Cross et al. [77] investigated the weekly load and risk of injury analysis of elite rugby union players across an English Premiership season. Cross et al. [77] found players were at a higher risk of training and match play injury when accumulated weekly loads (1245 AU) and week to week changes in training load (1069 AU) were significantly increased. The author[77] suggested that an increased risk of injury is potentially associated with physiological manifestation of

accumulated fatigue. The association between increases in accumulative training load and risk of injury has been previously identified in contact sports such as Australian Rules football [78, 80] and Rugby League [79]. No study has investigated the relationship between internal weekly training loads and the potential risk of injury or illness in Super Rugby competition. Previous study by Cunniffe et al. [15] has investigated the relationship between [s-IgA], URTI and weekly training loads in elite rugby players across a rugby competition. Cunniffe et al. [15] found intense duration periods in weekly loads in pre-season and in-season phases were associated with suppression in [s-IgA] and higher incidences in URTI in elite rugby union players. Cunniffe et al. [15] proposed increases in training loads elicit hormonal disturbances (increase in cortisol), which could contribute to the reduction in mucosal immunity. The suppression in s-IgA concentration during intense training and competition phases in a rugby season demonstrates the physiological [25], psychological [68], and environmental [69] stresses that influence the immunological system, leaving athletes with an increased risk of airborne and viral infections such as URTI. The present study will investigate whether variances in accumulated weekly training loads and week to week changes in weekly training loads of Super Rugby competition will influence the s-IgA response of elite rugby union players to Super Rugby match play.

Salivary analyses which provide information regarding changes in immunological markers such as immunoglobulin A, in conjunction with the monitoring of weekly training loads may give greater insight into the physiological disruption that occur in response to elite Super 15 match-play. Currently, limited research is available which investigates the immunological responses to training and match performances during in season periods of a Super 15 competition.

2.8 Analysis of Salivary Immunoglobulin A

Salivary analysis in applied research enables non-invasive efficient measures to be collected in a sporting environment. Previous literature in rugby union [15, 44] have investigated s-IgA, by utilizing a serum based Enzyme Linked Immunosorbent Assay (ELISA), which comprises of collecting passive drool of saliva to assays [63]. The salivary assays are then analysed using a buffering solution that contains the conjugate (such as horseradish peroxidase) and substrate (such as 3,3',5,5'-tetramethylbenzidin) needed to determine [s-IgA] [63]. The conjugate is an enzyme that is used to bind to a different location of the targeted antigen [63]. The binding of the conjugate enzyme to the targeted antigen, allows for the substrate to bind to the conjugate, which causes a reaction to yield a colour to the targeted antigen [63], which is measured via a spectrophotometer (plate reader) to determine the concentration of the targeted antigen [63]. The appearance of colour will influence whether antibodies are present (colour is absent) or absent (colour is present) [63]. Although the ELISA method is a reliable and valid method to detect immunological parameters, it is expensive and time consuming. The cost and the flexibility to collect data during match competition, makes it difficult for researchers to collect immunological

data in an elite sporting environment.

The Individual Profiling (IPRO) interactive saliva analysis system has been established for immune and endocrine analyses in an applied sports setting [46]. The advantage of utilising the IPRO is the ability to obtain non-invasive data and provide feedback within seven minutes. Validity and reliability tests of the IPRO device by Coad et al. [67] were conducted on recreational active males (n = 12) and females (n = 13). Reliability measures of the IPRO method were assessed by comparing the variation between two s-IgA samples measured co currently for each participant [67]. Validity measures of the IPRO method were assessed by comparing the [s-IgA] from the IPRO with a [s-IgA] from the ELISA method [67]. Results of the study by Coad et al. [67] found strong positive correlation ($r = 0.93$, $p < 0.001$), with no significant difference in [s-IgA] compared with the ELISA method, and also found reliable (ICC $r = 0.89$, $p < 0.001$ and CV = 9.40 %) for measures of [s-IgA]. Coad et al. [67] support the use of the IPRO to be a valid ($r = 0.93$, $p < 0.001$) and reliable ($r = 0.89$, $p < 0.001$ and CV = 9. 40 %) tool to measure s-IgA in professional rugby union players following match-play. The IPRO is therefore an effective monitoring tool to determine the [s-IgA] of professional rugby union players following match play.

Chapter Three: Methods

3.1. Participants

The study involved twenty-three elite rugby union players (mean ± standard deviation; age 24.8 ± 2.9 years; stature 185.6 ± 6.8 cm; weight 100.7 ± 11.2 kg). All players are full time athletes, playing in the Super 15 competition, with a mean Super 15 career of 56.3 ± 24.7 rugby games. Twenty-three players were selected to play for each game, with seventeen players involved in game one, sixteen players in game two and seventeen players in game three were included in the study. Ethical approval was obtained from the Bond University Human Research Ethics Committee (BUHREC). Reference number: RO1710 (Immunological Response of Rugby Union players to Super 15 Rugby match-play).

3.2. Experimental Design

Saliva samples were collected at 32 hours pre-match play, one-hour post-match play, and 32 hours post-match play across three Super 15 home matches (Match 10, Match 12, Match 15) during the Super 15 Rugby Season. Players were asked to refrain from strenuous exercise within the 24 hour period prior to the collection of baseline saliva samples. Participants were also instructed to consume their 'typical' diet 48 hours prior to rugby match-play and asked to avoid consumption of food or brushing their teeth in the two hours prior to the sample collection. Players were also asked to have had not consumed alcohol for at least 24 hours prior to sample collection. Players were required to play 40 minutes or more to be included in the study. Players were instructed to provide saliva samples within 1 hour post-match play prior to the ingestion of food and participation of post-match recovery modalities. Players were provided a day off post-match play and asked to refrain from strenuous exercise until the collection of saliva samples the following day prior to the commencement of training sessions (36 hours post-match play). Saliva samples were collected at the training and competition venue to minimise any disturbances in a controlled environment.

3.3. Procedures

3.3.1 Training and Game Load

Player weekly loads were collected across the Super Rugby season and were determined using the Borg category-ratio 10 scale [81, 92] and the total duration of training and match play. Players were required to provide a Rating of Perceived Exertion (RPE) within 30 minutes after the completion of a training session, see Table 1, for every session across the Super Rugby season. The RPE was multiplied with total duration time to determine weekly internal loads of players involved across the season. Session RPE scales are suggested to be a valid method for quantifying training loads in elite sports [81, 92], where strong correlations have been found with blood lactate concentration (r=0.86) and heart rate measures (r = 0.89) in elite rugby league players [85].

Rating	Description
0	Rest
1	Very, Very Easy
2	Easy
3	Moderate
4	Somewhat Hard
5	Hard
6	
7	Very Hard
8	
9	
10	Maximal

Table 1. Modified Rating of Perceived Exertion (RPE) Scale (Foster et al. 81).

Weekly player work load was calculated using the sum of internal load (RPE x Total Duration Time) over each 7 day period commencing from Monday. Two methods were used to assess changes in weekly load: a) week to week changes in player work load (absolute values) b) training stress balance where the player's acute load (workload across 7 days) is divided by the chronic (workload across 28 days) workload [77, 86].

3.3.2 Contact Involvements

Total contact involvements were collected across all three games through notational statistical analysis, where recorded match play were coded using an analytical software (Fair Play Pty Ltd, Brisbane, Australia). The sum of tackle, ball carry and ruck and maul involvements were calculated to determine the number of contact involvements in the game for every player. The definition of a tackle involves an event where the player was involved (within two metres radius of the ball) in the progress of halting an opponent who has the possession of the ball [93]. A ball carry is defined in the event of a player being tackled whilst maintaining the possession of the ball [93]. Ruck and maul involvements were defined as when a player was involved (within two metres radius of the ball) when a ruck and maul was formed (under the laws of the game [93].

3.3.3 Individual Profiling (IPRO) Saliva Analysis

3.3.3.1 Equipment

Saliva Analysis using the IPRO (Oxfordshire, UK) device contains an Oral Fluid Collector swab which consists of a synthetic polymer-based material attached to a volume adequacy indicator stem, a dropper bottle with extraction buffer, s-IgA Lateral Flow Device (LFD) and a IPRO LFD Reader [47]. The OFC synthetic polymer-based swab material provides a clear colour change (blue) when 0.5mL (± 20%) of passive saliva is collected. The swab will then be placed into the dropper bottle containing extraction buffer solution. The extraction buffer solution extracts the targeted analytes from the OFC swab into the buffer for preservation.

3.3.3.2 Procedure

Unstimulated saliva was collected from players using an Oral Fluid Collector (IPRO OFC, Ipro Interactive, Oxfordshire, UK), which consists of a synthetic polymer based material on a polypropylene tube. Players were required to abstain from food and fluids other than water in the 60 minutes before providing each saliva sample. Players were also required to wait for 10 minutes after their last consumption of water before collecting any saliva samples. The collection of saliva samples involved placing the OFC onto the tongue for for one to two minutes until the volume indicator of the swab turns blue. Salivary IgA (ug/mL) was analysed using a commercially available lateral flow immunoassay test kits (IPRO Lateral Flow Device (LFD), (IPRO Interactive Ltd., Oxfordshire, England). The immunochromatographic strip (ICS) test was conducted under the instructions from the manufacturer. The sample is shaken for two minutes. Once shaken, two drops from the buffered solution is placed onto the LFD Reader for s-IgA analysis. The liquid runs along the test strip through the conjugate pad being absorbed by the dried conjugate to indicate a control and test line visible in the test window. Five minutes after the sample is added onto the LFD, the test line is measured in an IPRO plate reader (IPRO Interactive Ltd., Oxfordshire, England) to provide quantitative feedback by converting the line intensity of the strip to the corresponding [s-IgA].

3.4. Statistical Analysis

All variables were assessed for parametric assumptions using the Shapiro Wilko Test. These variables included week to week changes in training load, week to week training Stress balance changes in training load, [s-IgA] across the three time points (pre 32 hours, post 1 hour, post 32 hours), and total contact involvements (sum of tackling and ruck involvements) across the three matches. Variables were log transformed due to non-uniformity of error. Variables were assessed using Will Hopkin's Effect Size (ES) with 95% confidence interval (CI) and percentage (%) change to determine the magnitude of changes between each time point for s-IgA, Training stress balance, and weekly training load [87]. Calculations were performed on an Excel spreadsheet, where the ES was determined using the following formula: (M1-M2)/s where M1 – mean of one group, M2 – mean of second group, s = standard deviation [87]. Magnitude of change was classified by the percentage of effect size, where if the value was greater than 75% likelihood or the value was greater than or equal to the ES ± 0.2, it was reported as a substantial increase or decrease [87]. If the value resulted in a smaller effect, the value was classified as trivial, unless the magnitude of change crossed the boundaries of the ES -0.2 and 0.2, where it was reported as unclear [87].

Total contact involvements were assessed for parametric assumptions, using the Shapiro Wilko Test, before performing a multifactorial analysis of variance (MANOVA) with repeated measures for all dependent variables. Where significant main effects for total contact involvements across the three matches occurred ($p < 0.05$), a Tukey post-hoc test was used to identify the source of the differences. A Pearson's correlation analysis was conducted between total contact involvements and the difference of [s-IgA] between pre 32 hours match play and post 1 hour match play.

Chapter Four: Results

The results of the present study found substantial increase in [s-IgA] between 32 hours pre and 1 hour post match play for forwards (49.3 ± 25.8 %, ES 1.29 ± 0.74) and backs (45.8 ± 22.6 %, ES 1.78 ± 0.96), and between 32 hours pre and 32 hours post match play for forwards (50.9 ± 38.9 %, ES 1.32 ± 1.06) and backs (23.1 ± 50.8 %, ES 0.98 ± 1.93) in Match 10, as observed in Table 2. The magnitude of change between 1 hour post match play and 32 hours post-match play was unclear for forwards (1.0 ± 38.0 %, ES 0.03 ± 1.03) and backs (-15.6 ± 64.5 %, ES -0.8 ± 2.34), as observed in Table 2.

In Match 12, the magnitude of change substantially increased between 32 hours pre and 32 hours post match play for forwards (40.3 ± 32.6 %, ES 0.89 ± 0.74) and backs (54.2 ± 45.2 %, ES 1.06 ± 0.92), and between 1 hour post match play and 32 hours post match play for forwards (53.1 ± 39.4 %, ES 1.12 ± 0.87), as observed in Table 2. The magnitude of change was unclear between 32 hours pre and 1 hour post match play for forwards (-8.3 ± 63.7 %, ES -0.23 ± 1.3) and backs (15.1 ± 122 %, ES 0.35 ± 1.96), and between 1 hour post match play and 32 hours post match play for backs (33.9 ± 105.6 %, ES 0.72 ± 1.77) as observed in Table 2.

During Match 15, the magnitude of change were unclear in [s-IgA] between 32 hours pre and 1 hour post match play for forwards (-3.2 ± 32.4 %, ES -0.1 ± 0.83) and backs (-17.3 ± 80.9 %, ES -0.82 ± 2.55), and between 32 hours pre and 32 hours post match play for forwards (14.5 ± 46.9 %, ES 0.4 ± 1.14) and backs (-13.3 ± 39.5 %, ES -0.62 ± 1.43), and between 1 hour post-match play and 32 hours post-match play for forwards (18.3 ± 49.7 %, ES 0.5 ± 1.2) and backs (4.8 ± 95.2 %, ES 0.2 ± 2.88), as observed in Table 2.

Table 2. Effect Size (ES) ± 95% Confidence Interval Change, Qualitative Descriptor and Percentage Change of Pre 32 Hours – Post 1 Hour Match Play, Pre 32 Hours-Post 32 Hours Match Play, Post 1 Hour-Post 32 Hours Match Play for Salivary IgA concentration (µg/mL) in Forwards and Backs across three Super 15 Rugby Matches. The qualitative descriptor was defined as a substantial increase or decrease when the ES ≥ 75% likelihood of the effect being ≥ the ES 0.2 (small) reference value. Effects were classified as unclear where the ±95% CI of the ES crossed the boundaries of ES -0.2 and +0.2 (Adapted from Cormack et al. [87]).

Super Rugby Matches	Forwards			Backs		
	Pre 32 Hours - Post 1 Hour Match Play	Pre 32 Hours - Post 32 Hours Match Play	Post 1 Hour - Post 32 Hours Match Play	Pre 32 Hours - Post 1 Hour Match Play	Pre 32 Hours - Post 32 Hours Match Play	Post 1 Hour - Post 32 Hours Match Play
Match 10	1.29 ± 0.74 Substantial ↑ 49.3 ± 25.8 %	1.32 ± 1.06 Substantial ↑ 50.9 ± 38.9 %	0.03 ± 1.03 Unclear 1.0 ± 38.0 %	1.78 ± 0.96 Substantial ↑ 45.8 ± 22.6 %	0.98 ± 1.93 Substantial ↑ 23.1 ± 50.8 %	- 0.80 ± 2.34 Unclear - 15.6 ± 64.5 %
Match 12	- 0.23 ± 1.30 Unclear - 8.3 ± 63.7 %	0.89 ± 0.74 Substantial ↑ 40.3 ± 32.6 %	1.12 ± 0.87 Substantial ↑ 53.1 ± 39.4 %	0.35 ± 1.96 Unclear 15.1 ± 122 %	1.06 ± 0.92 Substantial ↑ 54.2 ± 45.2 %	0.72 ± 1.77 Unclear 33.9 ± 105.6 %
Match 15	- 0.10 ± 0.83 Unclear - 3.2 ± 32.4 %	0.40 ± 1.14 Unclear 14.5 ± 46.9 %	0.50 ± 1.20 Unclear 18.3 ± 49.7 %	- 0.82 ± 2.55 Unclear -17.3 ± 80.9 %	- 0.62 ± 1.43 Unclear -13.3 ± 39.5 %	0.20 ± 2.88 Unclear 4.8 ± 95.2 %

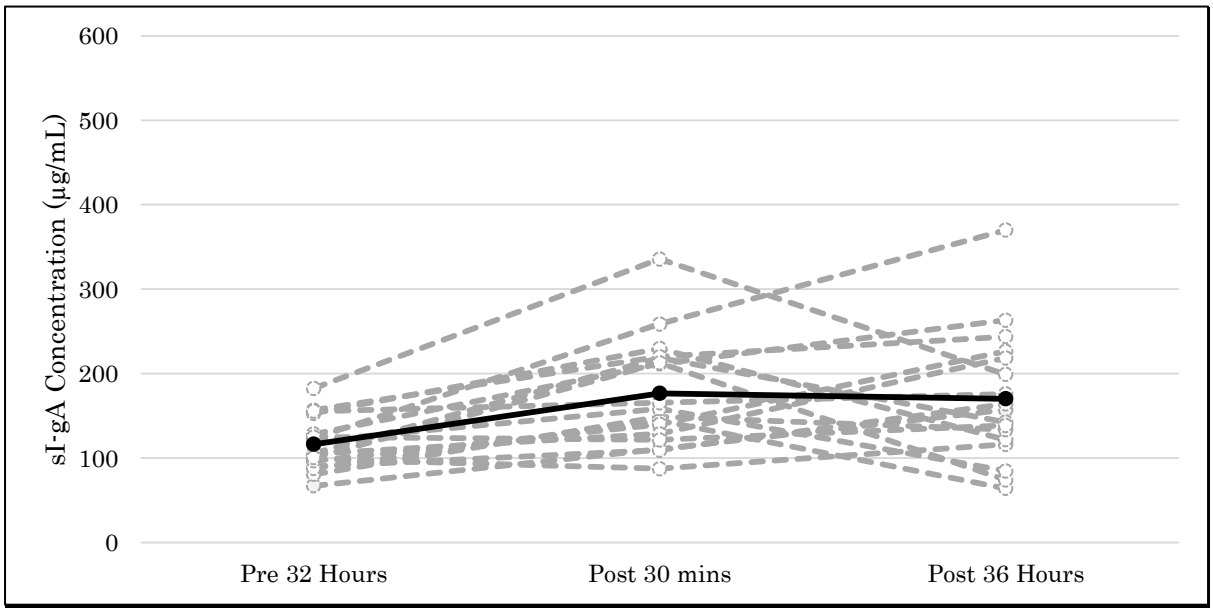

Figure 3. Salivary IgA Concentrations (µg/mL) of Elite Rugby Union Players at 32 Hours Pre Match Play, Post 1 hour Match Play and Post 32 hours Match Play in Match 10. Individual data are presented as open shapes and dashed lines and mean group values are presented as solid line and solid shapes.

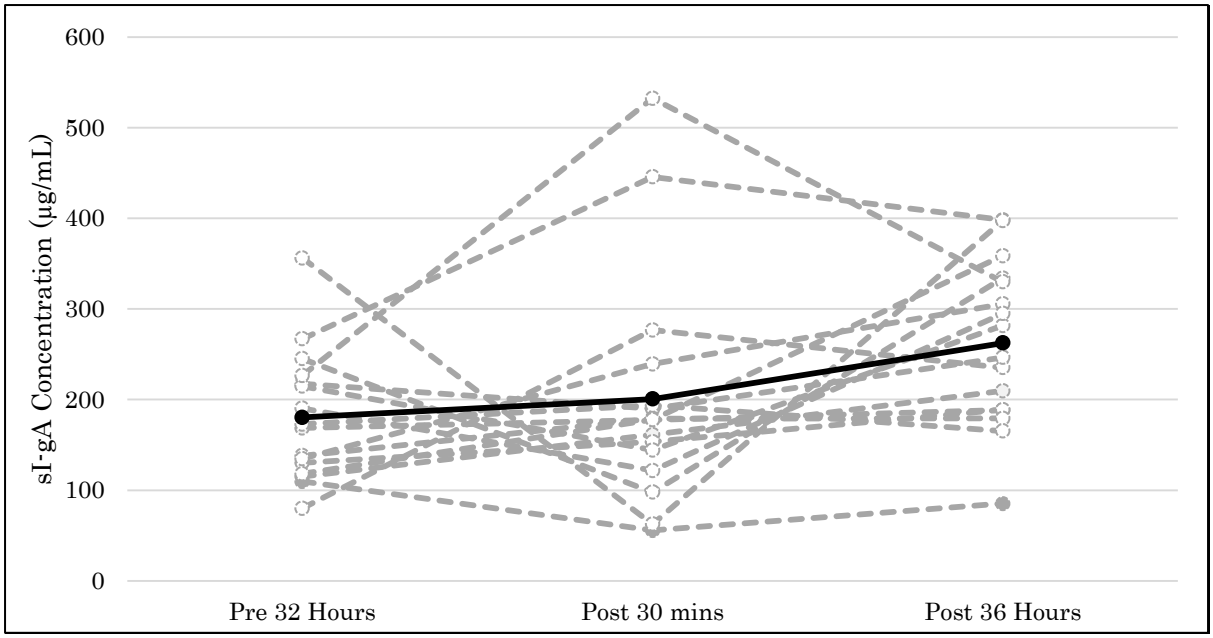

Figure 4. Salivary IgA Concentrations (µg/mL) of Elite Rugby Union Players at 32 Hours Pre Match Play, Post 1 hour Match Play and Post 32 hours Match Play in Match 12. Individual data are presented as open shapes and dashed lines and mean group values are presented as solid line and solid shapes.

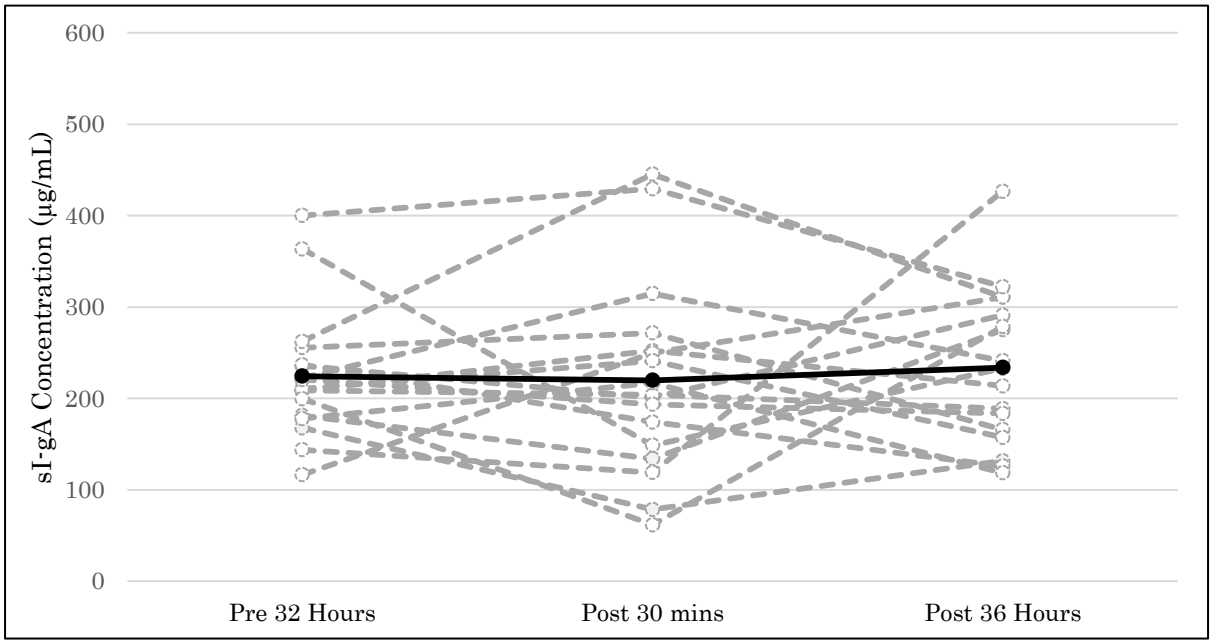

Figure 5. Salivary IgA Concentrations (µg/mL) of Elite Rugby Union Players at 32 Hours Pre Match Play, Post 1 hour Match Play and Post 32 hours Match Play in Match 15. Individual data are presented as open shapes and dashed lines and mean group values are presented as solid line and solid shapes.

Weekly training loads and training stress balance (TSB), which is calculated by acute workload (1 week of training load) divided by the chronic workload (rolling 4-week average training load) were analyzed across the Super Rugby season for backs (observed in Figure 6), and forwards (observed in Figure 7). Statistical analyses of weekly loads and training stress balance was conducted for the preceding week and the week of testing conducted (as observed in Table 3 and 4). The effect size of changes seen in week to week load (as observed in Table 3) found substantial decreases between Match 9 and Match10 for forwards (-37.5 ± 22.3%, ES -2.21 ± 0.95) and backs (-26.1 ± 12.1 %, ES -2.36 ± 0.92), and between Match 14 and Match 15 for forwards (-22.0 ± 30.0%, ES -1.10 ± 1.17) and backs (-19.1 ± 37.8%, ES -0.89 ± 1.35). The effect size of changes in Match 11 and 12 found substantial increases for forwards (14.8 ± 17.2%, ES 0.53 ± 0.61) and backs (23.8 ± 37.8%, ES 0.50 ± 0.75).

The effect size of changes seen for training stress balance (as observed in Table 4) found substantial decreases between Match 9 and Match 10 for forwards (-34.3 ± 15.8%, ES -2.07 ± 0.72) and backs (-34.8 ± 19.4%, ES -1.07 ± 0.44), and between Match 14 and Match 15 for forwards (-20.6 ± 27.4 %, ES -1.48 ± 1.55) and backs (-22.5 ± 36.6 %, ES -1.32 ± 1.62). The effect size of changes in Match 11 and 12 found substantial increases for forwards (15.7 ± 15.6%, ES 0.83 ± 0.82) and backs (20.5 ± 42.4 %, ES 0.56 ± 1.06).

Table 3. Effect Size (ES) ± 95% Confidence Interval Change, Qualitative Descriptor and Percentage Change of Match 9 - Match 10, Match 11 – Match 12, Match 14 – Match 15 for week to week loads in Forwards and Backs in the squad. The qualitative description is defined as a substantial increase or decrease was classified when the ES ≥ 75% likelihood of the effect being ≥ the ES 0.2 (small) reference value. Effects were classified as unclear where the ±95%CI of the ES crossed the boundaries of ES -0.2 and +0.2 (Adapted from Cormack et al. [87]).

Super Rugby Matches	Forwards	Backs
Match 9-10	-2.21 ± 0.95 Substantial ↓ -37.5 ± 22.3%	-2.36 ± 0.92 Substantial ↓ -26.1 ± 12.1 %
Match 11-12	0.53 ± 0.61 Substantial ↑ 14.8 ± 17.2%	0.50 ± 0.75 Substantial ↑ 23.8 ± 37.8%
Match 14-15	-1.10 ± 1.17 Substantial ↓ -22.0 ± 30.0%	-0.89 ± 1.35 Substantial ↓ -19.1 ± 37.8%

Table 4. Effect Size (ES) ± 95% Confidence Interval Change, Qualitative Descriptor and Percentage Change of Match 9 - Match 10, Match 11 – Match 12, Match 14 – Match 15 for Training Stress balance in Forwards and Backs in the squad. The qualitative description is defined as a substantial increase or decrease was classified when the ES ≥ 75% likelihood of the effect being ≥ the ES 0.2 (small) reference value. Effects were classified as unclear where the ± 95%CI of the ES crossed the boundaries of ES -0.2 and +0.2 (Adapted from Cormack [87]).

Super Rugby Matches	Forwards	Backs
Match 10 vs 9	-2.07 ± 0.72 Substantial ↓ -34.3 ± 15.8%	-1.07 ± 0.44 Substantial ↓ -34.8 ± 19.4%
Match 12 vs 11	0.83 ± 0.82 Substantial ↑ 15.7 ± 15.6%	0.56 ± 1.06 Substantial ↑ 20.5 ± 42.4 %
Match 15 vs 14	-1.48 ± 1.55 Substantial ↓ -20.6 ± 27.4 %	-1.32 ± 1.62 Substantial ↓ -22.5 ± 36.6 %

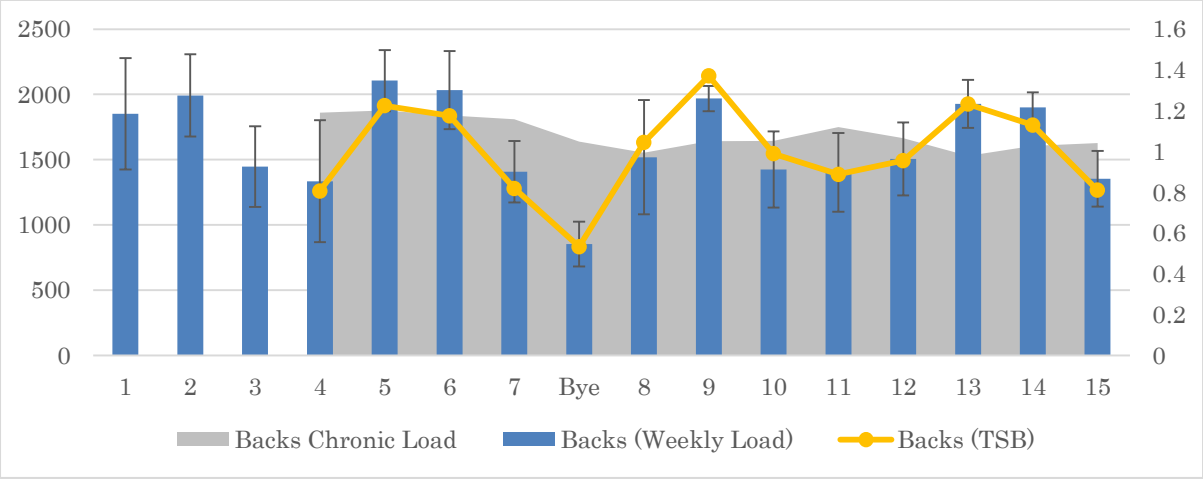

Figure 6. Weekly Training (AU) and Training Stress Balance (%) in Elite Rugby Union Players (Backs) across the Super 15 Rugby Season. Values are represented as mean ± SD. Salivary IgA was collected in Match 10, 12 and 15.

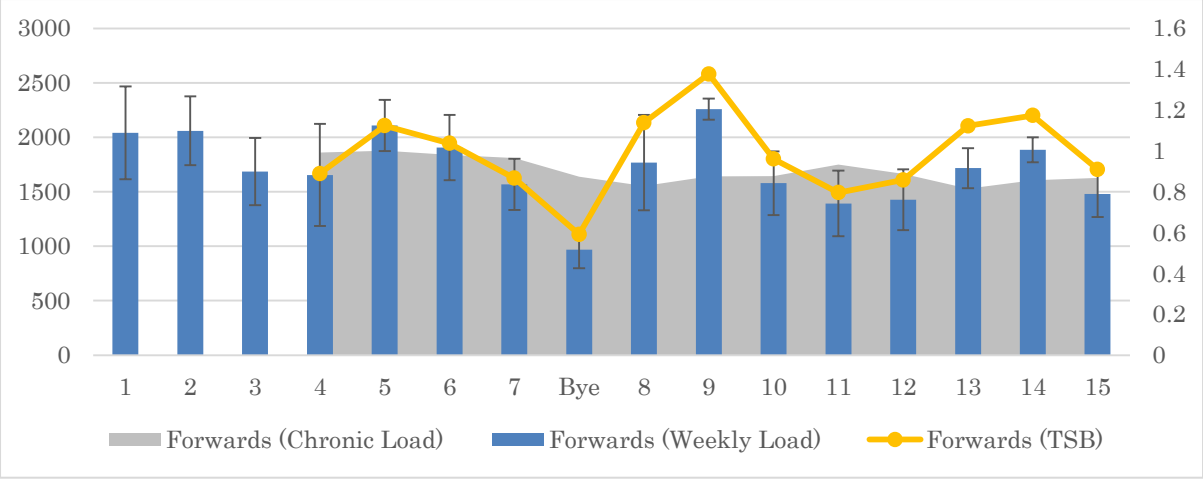

Figure 7. Weekly Training (AU) and Training Stress Balance (%) in Elite Rugby Union Players (Forwards) across the Super 15 Rugby Season. Values are represented as mean ± SD. Salivary IgA was collected in Match 10, 12 and 15.

Total contact involvements were compared across Match 10, Match 12 and Match 15 (as observed in Figure 8). Using MANOVA, there was a significant increase ($p<0.05$) between Match 10 (17.9±10.6) and Match 15 (9.1±5.4). No significant differences were found ($p>0.05$) between Match 10 (17.9±10.6) and Match 12 (12.4±6.7), and between Match 12 (12.4±6.7) and Match 15 (9.1±5.4). No correlations were found between total contact involvements and the difference of [s-IgA] between pre 32 hours match play and post 1-hour match play ($r=0.2$) (as observed in Figure 9).

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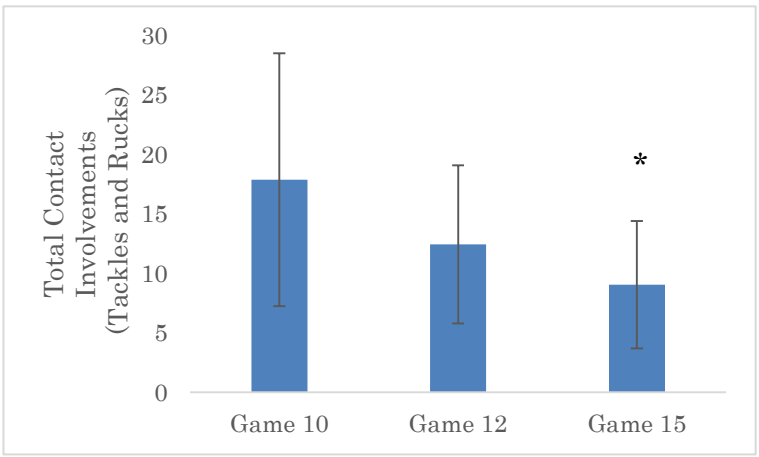

Figure 8. Total Contact Involvements of Elite Rugby Union Players for Match 10, Match 12 and Match 15 of the Super Rugby Season. *P<0.05 from Match 1.

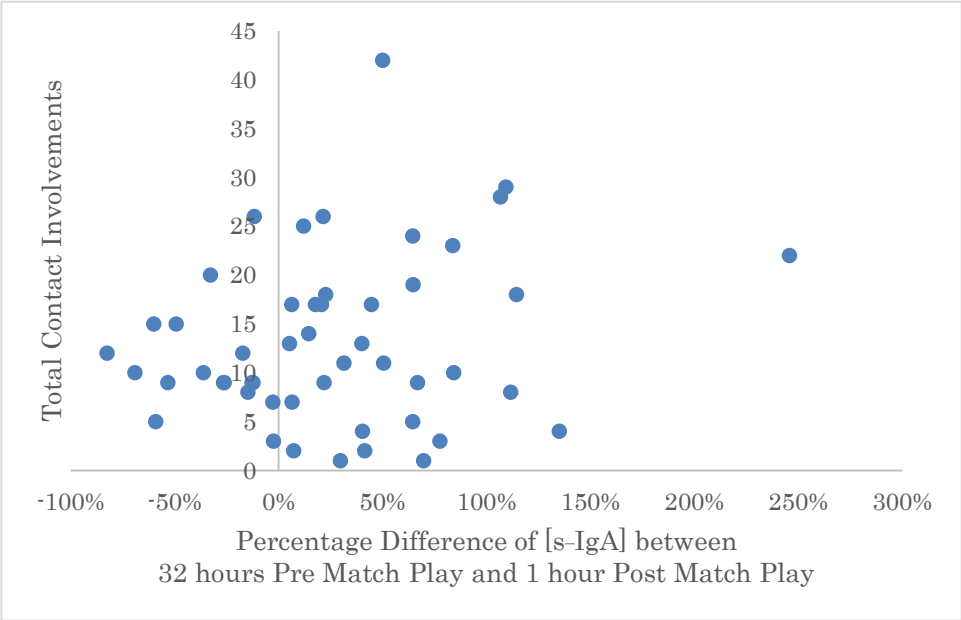

Figure 9. Relationship between total contact involvements and the percentage difference (%) of [s-IgA] between 32 hours pre-match play and 1 hour post-match play among elite rugby union players (r = 0.2).

Chapter Five: Discussion

The aim of the present research was to examine the s-IgA response of elite rugby union players to the physical demands of Super Rugby match-play over the course of three Super 15 rugby matches, and the incorporation of weekly 'in-season' load during a Super Rugby season. The present research found the s-IgA response to the demands of Super Rugby match play were heterogeneous across all three matches included in the study. There were substantial increases in [s-IgA] post-match play in Match 10 for both backs and forwards, and no significant disturbances seen in Match 12 and Match 15 for both backs and forwards. The present study also found immunological disturbance can last up to 32 hours post rugby match play, as seen in Match 10 and Match 12. The variation in s-IgA response post rugby match play and the prolonging of immunological disturbance up to 32 hours post-match play highlight the individual variations in response to rugby match play, and the importance of monitoring players and the subsequent prescription of recovery strategies and modulations in player training loads to ensure players are fully recovered from the physiological demands of rugby match play.

The study also found decreases in week to week changes and training stress balance changes for player workload when s-IgA samples were collected in Match 10 and Match 15. The decreases in weekly work load could potentially have an effect in minimizing the suppression of immune function. As a result, strategic periodization in player loading needs to be considered to reduce the risk of suppression in the immune function and subsequent risk of contracting URTI.

5.1 Salivary IgA Response to Super Rugby Match Play

The s-IgA response of Super Rugby match-play across three Super Rugby matches in elite rugby union players found no significant differences in [s-IgA] post rugby match play (Figures 3 to 5). The result of the present research is consistent with Koch et al. [44] who found similar trends in post-match response of [s-IgA] after a collegiate rugby match, and suggested the potential cause of non-significant changes in [s-IgA] may be due to within-subject variation of match demand, the influence of fluid intake, sweat rate and the small sample size in the study. The variation in the s-IgA response of elite rugby union players to rugby match play highlights the need to analyze the immunological responses of rugby match play on an individual basis. A previous study by Cunniffe et al. [6] in international rugby matches suggested that the potential mechanism for the variations of immunological response in rugby match play may be dependent on the different positional demands of rugby, physical capacity and the psychological stress of individuals. In the present research, the difference in positional demands in rugby union match play such as greater volume and high speed running demands in backs [3 – 6, 70], and the greater collision profiles in forwards [3 – 6, 70] will influence how s-IgA in elite rugby union players responds to Super rugby match play. The group of elite rugby players participating in the present research had a mean Super Rugby career of 56.3

± 24.7 Super Rugby matches. The repetitive exposure to Super rugby competition may enable elite rugby union players to cope with the physiological and psychological stress of rugby match play at an elite level.

The variation in [s-IgA] in elite rugby union players post rugby match play may be regulated by the sympathetic and parasympathetic drive of the autonomic nervous system (ANS) in response to the internal and external stressors of Super Rugby match play. The proposed mechanism is consistent with Cunniffe et al. [6] who suggested the regulation of the immune system is driven by the sympathetic response of the autonomic nervous system (ANS) and the hypothalamic pituitary adrenal axis (HPA). The substantial increase of [s-IgA] seen after Match 10 may be caused by the sympathetic drive of the Hypothalamic-Pituitary-Adrenal (HPA) axis in response to rugby match play. Previous studies have found post-exercise [s-IgA] to increase in soccer [16], and basketball matches [40]. The physiological nature of rugby union exhibits high intensity activities such as repetitive acceleration and deceleration, high speed running, sprinting and repetitive collisions. When homeostatic disturbances occur from internal and external stressors as seen in the high physiological demands of rugby, sympathetic innervation of the ANS and HPA axis may induce the release of catecholamine and glucocorticoids [6]. The release of neurotransmitters such as catecholamine and glucocorticoids, stimulates the increase expression of s-IgA in pIgR and activate the facilitation of transporting s-IgA complexes across the endothelial cells to enter the blood stream [12, 29,83, 84]. The influx of [s-IgA] post-match play may also be caused by the neural stimulation of the local immune system, where the activation of lymphoid organs can stimulate immune cells to secrete and produce local s-IgA [83, 84].

The insignificant differences in Match 12 and Match 15 post-match play suggests alterations within the subject's immune system. A previous study by Cunniffe et al. [15] has found decreases in [s-IgA] have been associated with inadequate recovery and increases in training volume and match play demands in the sport of rugby [15]. The suppression of [s-IgA] may be due to the down regulation of the endocrine response to chronic and high intense exercise [29,30,49]. Alterations in the Hypothalamic-Pituitary-Adrenal (HPA) axis will inhibit the synthesis of s-IgA and down regulate pIgR synthesis [29, 30]. The down regulation in pIgR decreases the transportation of s-IgA, which decreases [s-IgA] at the mucosal level [29, 30]. The group of elite rugby players participating in this study has a mean Super Rugby career of 56.3 ± 24.7 Super Rugby matches. The extensive experience and repetitive exposure to the physical demands during intensive training phases and previous Super Rugby matches in previous seasons may promote physiological adaptations to enhance coping in regards to the demands of rugby match play. The repetitive exposure to rugby match play at an elite level may have minimized the immunological disturbances, as seen in previous studies [6, 14, 15].

Match 10 and Match 12 demonstrated further immunological disturbances post-match play recovery, with

substantial increases in [s-IgA] observed between 32 hours pre-match play and 32 hours post-match play. The substantial increase in [s-IgA] 32 hours post-match play in this present research suggests immunological disturbance still occurs during the recovery period post rugby match play. The proposed mechanism is consistent with study by Cunniffe et al. [6] who also found a delay in immunoendocrine markers such as neutrophil, T lymphocytes, NK cells, interlukin-6 back to baseline values 36 hours post-match play in international rugby [6]. Cunniffe et al. [6] suggests the delay in physiological responses in the endocrine and immune systems is a physiological "rebound" effect to the physiological disruptions occurring during rugby match play. Cunniffe et al. [6] also suggested the immune system may enter into a refractory state following post rugby match play, which can cause the delayed in the immune response to return to baseline measures. The immune system being in a refractory state could potentially explain for the increase in [s-IgA] 32 hours post-match play where a second rebound effect may occur in response to the suppression of the immune system post rugby match play. However, the limitation to the current study was the ability to collate [s-IgA] between the 1 hour and 32 hours post-match play to suggest if there was a suppression in [s-IgA] after the immediate increase of [s-IgA] following rugby match play.

5.2. Immune Response in relation to Total Contact Involvements

The present research found significant increases ($p < 0.05$) in total contact involvements in Match 10 compared to Match 15. Previous studies by Smart et al. [71] and Takarada et al. [53] found increases in the number of tackles and time spent defending during rugby match play has been suggested to be associated with an increase in tissue trauma [53, 71]. The physiological disruptions within the tissue stimulates the infiltration of neutrophils and monocytes to commence cellular repair and regeneration [88]. The influx of neutrophils and monocytes is initiated via the activation of inflammatory markers, which initiates the stimulation of immunological markers [6, 88]. The activation of inflammatory markers in response to the repeated bouts of heavy collisions in rugby match play, stimulates the rebound response of the immune system, where up-regulation of immunological markers such as s-IgA, T lymphocytes, NK cells are transferred and released to mitigate the inflamed area [6, 88]. The physiological rebound effect in response to tissue trauma could potentially explain the increase [s-IgA] with an increase in total contact involvements in Match 10. However, the mechanism is difficult to propose as findings in this study showed no correlation between the number of contact involvements and the percentage differences in 32 hours pre match and 32 hours post-match play. Interstitial markers such as Creatine Kinase (CK) was also not measured in this study. Future research needs to investigate the relationship between s-IgA, inflammatory markers (cortisol and interlukin-6) [6], interstitial markers (CK) [53, 71] and the number of contact involvements across a Super Rugby season to determine whether there is a rebound effect in response to tissue trauma in Super Rugby match play. Such data would be valuable to have a greater understanding on whether greater contact involvements in match play will have an influence on immune function and the subsequent prescriptions

for training load and recovery management.

5.3. Immune Response in relation to Week to Week Changes and Training Stress Balance

Strategically planned periodization where optimal training load is sustained within a target threshold zone across a competitive fixture may be an important tool in reducing the risk of illness and injury in team sports [77, 78]. Chronic and acute changes in weekly workloads have been found to influence the regulation of the immune system, where an increase in weekly workloads haven been associated with incidences of illness in elite rugby union players across a competitive rugby season [6, 77]. In the present research, substantial decreases in week to week training loads and training stress balance were observed between Match 9 and Match 10, and Match 14 and Match 15 for forwards and backs. The substantial decreases in week to week workloads and training stress balance also coincide with the significant increase in [sIgA] post rugby match play in Match 10 and no significant difference in [sIgA] post rugby match play in Match 15. The reduction in week to week training load and training stress balance may influence rugby union players to be adequately recovered from accumulated weekly workloads, which may minimize the immunological suppression in response to the physiological disturbance of rugby match play. Careful tapering in weekly workload may potentially be an effective strategy to minimize the physiological disturbances and restore immunological function and mucosal immunity seen in team sports [89]. Previous studies by Moreira et al. [90], found tapering phases in a futsal competitions demonstrated significant decreases in the incidence of URTI in futsal players. Moreira et al. [90] also found negative correlations in the [s-IgA] and player weekly load.

In the present research, substantial increase in week to week workloads and training stress balance were observed between Match 11 and Match 12, for forwards and backs. The increase in weekly workload coincided with the insignificant differences of [sIgA] post-match play in Match 12, which suggests alterations within the subject's immune response to rugby match play. The increase in week to week training load could lead to rugby players accumulating fatigue, where there is a reduction in the amount of stress the physiological body can tolerate and increases the risk for injury and illness [77]. The chronic sympathetic innervation and chronic inflammation during intense exercise have been found to down-regulate the expression of s-IgA in the mucosal system [15]. The decrease in host immunity leaves rugby union players at an increased risk of contracting URTI post rugby match play [15]. Previous studies have also found that increases in weekly training load are associated with immunological suppression in elite athletes as seen in American football [39], rugby union [15] and soccer [90]. International and English rugby tournaments [15, 77] have found that increases in the chronic, week to week training load (> 1069 AU) and training at high intensities (cumulative 4 week loads > 8651 AU) have been associated with an increased risk for injury and illness in elite rugby players. The present research suggests the importance of player load management, where minimizing substantial increases in player weekly load, whilst

sustaining optimal loads can reduce the potential to suppress host immune function and the risk of illness and injuries in elite rugby union players.

Chapter Six: Conclusion and Practical Implications

The present research aimed to investigate the immunological response induced by Super Rugby match play, where it was found that responses in [s-IgA] were heterogeneous across all three time points in all three Super Rugby matches. There were substantial increases in [s-IgA] post-match play in Match 10 for both backs and forwards, and no significant disturbances seen in Match 12 and Match 15. The variation in s-IgA response post rugby match play indicate how physical capacity, positional demands and Super Rugby experience can influence how elite rugby union players respond to the physiological demands of Super Rugby match play. These inconsistencies in the study's findings highlight the individual variations in response to rugby match play, and the importance of monitoring players on an individual basis. The increase in [s-IgA] in Match 10 could be attributed to the immune rebounding effect from the physiological disruptions seen in rugby match play, where the physiological demands of rugby union initiate the response of sympathetic neural drive to stimulate the proliferation of immune markers. The present study also found significant increases in [s-IgA] during recovery periods (32 hours post-match play), which suggests the host immunity may not fully restore to its baseline values. The increase in [s-IgA] during the recovery period highlights the importance of recovery strategies and modulations in player training loads to ensure players are fully recovered from the physiological demands of rugby match play.

The study also found decreases in weekly training loads when s-IgA samples were collected in Match 10 and Match 15. The decreases in weekly training load could potentially have an effect in minimizing the suppression of immune function. As a result, strategic periodization in player loading needs to be considered, and where appropriate, de-loading phases should be potentially utilized to reduce the risk of suppression in the immune function and subsequent risk of contracting URTI. Future studies are required to investigate on the immune responses across the Super Rugby season, particularly considering traveling factors and accumulation of loads.

The implications of the present study suggest the following:

- The importance in combining methods of portable salivary measures, such as IPRO to examine [s-IgA] and the analysis of player work load (RPE x time) to determine how the immunological system of elite rugby union players respond to the physical workloads and demands of Super Rugby match play.

- The ability to collate immunological samples and provide immediate feedback, such as the IPRO, is a readily available and potentially meaningful tool to identify athlete physiological or immunoendocrine status and make immediate decisions with respect to training management to reduce the risk of contracting infections such as URTI among elite rugby union players.

- Analyses of individual player loading across the entire season is important in determining if a player is at high risk of injury and illness from an overload in training and match play workloads.
- Increased understanding of the high physical workload required for the Super Rugby season can be utilised to apply strategies to ensure players are able to adapt and accommodate to the physiological demands of Super rugby match play.

Chapter Seven: Future Research

Based on the findings in this study, the following recommendations are proposed for future investigation.

7.1. Measuring s-IgA in a Super Rugby Season

The aim of the present study was to observe the acute immune response of Super Rugby match play. The Super Rugby season presents players with unique logistical and performance challenges across 20 home and away matches and 3 weeks of finals. Future longitudinal studies need to investigate the immunological response of elite rugby union players to the physiological and psychological stresses of international travel, vast changes in environmental conditions (e.g. variations in humidity, altitude, training and playing surfaces) and the effects of cumulative fatigue associated with weekly regular season matches.

7.2 Measuring External Loads across the Super Rugby Season

Global Positioning System and Integrated Accelerometry has previously been used to determine match play demands in Super Rugby [70]. In this study, the lack of compliance and availability of GPS units made it difficult to collate external loads in elite rugby union players. Future studies need to investigate the external loading of elite rugby union players through the use of GPS micro-technology during a Super Rugby season. The ability to collate training and match play loads can be utilized to observe if there are correlations between internal training load (session RPE multiplied by duration), external training load (GPS micro-technology), [s-IgA], and the incidences of URTI. Utilising GPS and Integrated Accelerometry micro-technologies also helps determine whether changes in variables such as high speed distances, collisions and repeated accelerations and decelerations has an influence on the immunological response of elite rugby union players to Super Rugby match play and seasonal demands of Super Rugby. Optimal loads could also be determined as suggested in previous studies [77].

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Appendices

Appendix A. Informed Consent Form

Faculty of Health Sciences and Medicine

INFORMED CONSENT FORM

Chief Investigator(s): Name(s): Sean Yoshiura
Faculty: Health Sciences and Medicine
Contact number(s): 0408 193 052
Supervisor: Associate Professor Chris McLellan

Project Title: Acute Immunological Response of Elite Rugby Union players in a Super 15 match

(I) I have read the explanatory statement for the research project "Acute Immunological Response of Elite Rugby Union Players in a Super 15 match" and clearly understand the content, and what is being asked of me as a volunteer in the experiment / project.

(II) The risks associated with my participation in the experiment have been clearly explained to me and I clearly understand the risks involved in my participation in the experiment / project "Acute Immunological Response of Elite Rugby Union Players in a Super 15 match".

(III) I have been told and accept the potential benefits of my participation in the experiment / project "Acute Immunological Response of Elite Rugby Union Players in a Super 15 match"

(IV) I have had every opportunity to ask questions with regards to the experiment / project "Acute Immunological Response of Elite Rugby Union Players in a Super 15 match" and the questions I have asked have been answered to my satisfaction. I also

understand that I can ask questions about the experiment / project and my participation in the experiment at any time.

(V) I understand that my records will be managed in a confidential manner and that any reporting of my personal results will be anonymous or included together with the results of other participants, as an average result for the group of volunteers.

(VI) I clearly understand what is being asked of me to participate in the experiment / project and I understand the risks and benefits of my participation in the experiment / project "Acute Immunological Response of Elite Rugby Union Players in a Super 15 match" and I agree to participate as a volunteer.

(VII) I understand that I can withdraw from the experiment / project "Acute Immunological Response of Elite Rugby Union Players in a Super 15 match" at any time without ramifications or guilt or aggravation from the investigators.

(VIII) I understand that at the appropriate time, I will be provided feedback on my performance in the experiment / project "Acute Immunological Response of Elite Rugby Union Players in a Super 15 match".

(IX) I understand that the experiment / project will be carried out as described in the explanatory statement, a copy of which I have retained. I understand that whether or not I decide to participate is my decision and will not affect my relationships with Bond University.

(X) I give my consent to participate in the experiment "Acute Immunological Response of Elite Rugby Union Players in a Super 15 match".

Signatures: …….. ……

Investigator(s) Date

…….. ……

Participant Date

…….. ……

Witness Date